Applicant: Peter M. Glazer and Pamela Havre

Serial No.: 09/783,338 Art Unit: 1637

Filed: February 14, 2001 Examiner: Jeffrey Norman Fredman

For: *"CHEMICALLY MODIFIED OLIGONUCLEOTIDE FOR SITE-DIRECTED MUTAGENESIS"*

Assistant Commissioner for Patents
Washington, D.C. 20231

APPEAL BRIEF

Sir:

This is an appeal from the final rejection of claims 6-14 in the Office Action mailed January 16, 2003, in the above-identified patent application. A Notice of Appeal was mailed on April 16, 2003. A check in the amount of $160.00 for the filing of this Appeal Brief for a small entity is also enclosed. An Associate Power of Attorney is also enclosed.

It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-1868.

(1) REAL PARTY IN INTEREST

The real party in interest is Yale University, the assignee.

(2) RELATED APPEALS AND INTERFERENCES

This is a continuation of U.S.S.N. 08/083,088 ('088). The claims in '088 were rejected on the basis that they were not enabled under 35 U.S.C. 112, for *in vivo* as well as in vitro use.

The Board of Appeals upheld the Examiner's rejection on the basis that the appellants had failed to provide evidence that the method could be used *in vivo* as well as *in vitro*. Evidence showing *in vivo* as well as additional evidence of *in vitro* efficacy was obtained after filing of the appeal, but could not be considered in the appeal. The present application was filed so that such evidence could be considered (submitted in the form of a Declaration under 35 U.S.C. 1.132). **The same evidence was filed in a related application, which issued as U.S. Patent No. 6,303,376, claiming a triplex forming oligonucleotide which does not include a mutagen, effecting mutagenesis by virtue of an extremely high affinity to the double stranded substrate instead, but having identical issues as to enablement for *in vitro* and *in vivo* utility.**

(3) STATUS OF CLAIMS ON APPEAL

Claims 6-14 are pending and on appeal.

(4) STATUS OF AMENDMENTS

Claims 6-14 have not been amended.

(5) SUMMARY OF THE INVENTION

The claimed methods specifically mutate a target region of a nucleic acid *via* the steps of a) hybridizing a mutagenic oligonucleotide to a target region of a double-stranded nucleic acid molecule (page 10, lines 21-25), wherein the mutagenic oligonucleotide comprises a mutagen incorporated into a single-stranded nucleic acid (page 4, lines 4-7) that forms a triple-stranded nucleic acid molecule with the target region (page 4, lines 7-9); and b) mutating the double-stranded nucleic molecule (page 4, lines 11-12) (also see page 10, lines 12-19). The mutagen

may be activated prior to mutating the double stranded nucleic acid (page 12, lines 5-8). The mutagen may be psoralen or acridine orange and be activated by light; an alkylating agent, a cis-platinum analog, a hematoporphyrin, a hematoporphyrin derivative, mitomycin C, a radionuclide, or a molecule that interacts with radiation to become mutagenic (page 12, lines 12-21). The mutation may alter the activity of the double stranded nucleic acid molecule (page 4, lines 12-14). The double stranded nucleic acid molecule may be all or part of a viral genome (page 13, lines 27-31) or a gene (page 4, lines 19-26). The gene may be an oncogene (page 4, lines 19-21) or a defective gene (page 10, lines 25-32). Each of the foregoing limitations can be found in the claims as originally filed.

(6) ISSUES ON APPEAL

The issue presented on appeal is whether claims 6-14 are enabled as required by 35 U.S.C. § 112, first paragraph.

(7) GROUPING OF CLAIMS

The claims do not stand or fall together. Claim 6 is drawn to a method for site-directed mutagenesis involving triple strand formation between a mutagenic oligonucleotide and a target double stranded DNA. Claims 7-9 define the method for site-directed mutagenesis of claim 6 with a selected mutagen. Claims 10-14 define the method for site-directed mutagenesis of claim 6 where the target DNA is a particular kind of double-stranded nucleic acid molecule. Reasons for the separate patentability of these groups of claims are provided below.

(8) ARGUMENTS

(a) The Claimed Invention

A long standing problem in the field of gene therapy has been how to selectively mutate a gene in the cell, not after isolation, but within the physiological milieu. Antisense technology has been used to modify expression, but not to induce specific changes. Homologous recombination has been used to induce specific changes but the likelihood of an error remains high. There are a number of specific gene defects that if corrected could correct the disorder. An example is hemophilia. The claims on appeal define a method for inducing specific mutations of double stranded DNA using triplex forming oligonucleotides.

The triplex-forming oligonucleotides are generally described at pages 10-11; mutagens are generally described at pages 11-12, citing numerous publications which describe the mutagens, their specificity, and how they are used. Many mutagens are commercially available and their specificity well characterized. The triplex-forming oligonucleotide is the "address label" for the mutagen to be delivered (i.e. the oligonucleotide gives specificity to the mutagen and directs it to its proper target sequence).

The claims do not specify where the genes are that are to be mutated. The genes may be *in vitro* in a cell free solution. The genes may be in isolated cells, for example, obtained by biopsy, from a bone marrow sample, or from blood. The genes may be in cells in tissue or a patient. Methods of administration to a patient is described at page 12, including suitable carriers, in most cases those which are currently in use for antisense oligonucleotide clinical trials. The specification at pages 12-13 discloses that the mutagenic oligonucleotides are dissolved in a physiologically-acceptable carrier, such as an aqueous solution or are incorporated within liposomes, and the carrier or liposomes are injected into the organism undergoing genetic manipulation, such as an animal requiring gene therapy or anti-viral therapeutics. The preferred route of injection is intravenous. Accordingly, one of ordinary skill in the art would be able to practice the claimed method of mutagenesis based on the specification.

Examples in the application demonstrate *in vivo* administration to isolated cells. Example 4 uses triplex forming oligonucleotides to target SV40 DNA transfected into monkey cells. The triplex forming oligonucleotides targeted to the SV40 DNA included a mutagenic molecule, psoralen, which effected a specific mutation at the targeted site. UV irradiation was used to activate the psoralen to form adducts at that site. Replication of the viral genomes in the monkey cells fixed the adducts into permanent mutations. The results demonstrate that targeted mutagenesis occurs more efficiently in mammalian cells (6% of SV40 genomes incurred targeted mutations) than in bacteria (0.2%).

Additional studies demonstrating the delivery of triplex forming oligonucleotides to mice, with uptake into the tissues of the animals were provided in the Declaration under 37

C.F.R. § 1.132 of Dr. Glazer submitted on November 15, 2002. These studies demonstrate the substantial uptake of triplex forming oligonucleotides in a number of tissues, using the same methods and reagents as described in the application.

(b) Rejections Under 35 U.S.C. § 112

i. The Legal Standard

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art, without undue experimentation (*See, e.g., Genentech, Inc. v. Novo Nordisk A/S*, 108 F3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993); See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v. Telectronics, Inc.,* 857 F.2d 778 (Fed. Cir. 1988); *In re Stephens,* 529 F.2d 1343 (CCPA 1976)). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation (*M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985)). In addition, as affirmed by the Court in *Spectra-Physics, Inc. v. Coherent, Inc.,* 827 F.2d 1524 (Fed. Cir. 1987), a patent need not teach, and preferably omits, what is well known in the art.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir. 1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the

quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors involved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation 'must not be unduly extensive.' *Atlas Powder Co., v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). There is no requirement for examples.

ii. The Legal Standard with Regard to Post-Filing Art

There is no legal requirement that an inventor have actually reduced the invention to practice prior to filing. MPEP at § 2164.02, *citing* Gould v. Quigg, 822 F.2d 1074 (Fed. Cir. 1987). "The specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation." *Id.* As discussed below, appellants demonstrate in the application that they have conceived and reduced to practice as of the date of filing the claimed invention in isolated cells. The examiner has questioned enablement for delivery to intact animals.

Evidence of reduction to practice in intact animals was submitted in the form of a Declaration. This data was obtained after filing of the application. This data can be submitted to prove the truth of the statements in the application. As the CAFC stated in In re Brana, 51 F.3d

1560, 1567 n.19 (Fed. Cir. 1995), a post-filing date declaration setting forth test results substantiating utility "pertains to the accuracy of a statement already in the specification. . . . It does not render an insufficient disclosure enabling, ***but instead goes to prove that the disclosure was in fact enabling when filed***" (emphasis added). An important distinction has been made by the Courts between evidence of the knowledge and ability of those of skill in the art at the time of filing and evidence to prove that statements made in the application are correct. In the former case, of course, only evidence which existed prior to the filing of the application, or evidence that certain knowledge existed at the time of filing, is admissible (In re Hogan, 194 USPQ 527 (CCPA 1977)). **In the latter case, as in this case, any evidence, developed at any time, may be submitted for consideration.**

The clearest affirmation of the seasonability of factual evidence developed after the filing date of an application is provided by the Court in In re Marzocchi (169 USPQ 367, 370 (CCPA 1971)). In discussing rejections under 35 USC 112 where an examiner asserts that the unpredictability of the art creates a reasonable doubt as to the accuracy of a particular broad statement (in the application) supporting enablement, the Court states:

> Most often, additional factors, such as the teachings of pertinent references[*], will be available to substantiate any doubts that the asserted scope of enablement is in fact commensurate with the scope of protection sought and to support any demands based thereon for proof.
>
> ***

> Not necessarily *prior* art references, it should be noted, since the question would be regarding the *accuracy* of a statement in the specification, not whether that statement had been made before. [emphasis in the original]
>
> *Id. at 367*

In *In re Wilson* (135 USPQ 442, 444 (CCPA 1962)), the Court agreed that a reference, published after the filing date of the application, was properly cited to show a state of fact. In *In re Langer* (183 USPQ 288, 297 (CCPA 1974)), the Court again noted that later published references "are properly cited for the purpose of showing a fact." In *In re Rainer* (134 USPQ 343, 345 (CCPA 1962)) the Court found no error in the limited use made of a reference published after Appellant's filing date to show a fact. While all of these cases involved publications cited by the Patent Office in support of rejections, the same standard applies to evidence cited by Appellant. See In re Hogan.

iii. Rejection of Claims 6-14 under 35 U.S.C. § 112, first paragraph

Independent claim 6 (from which claims 7-14 depend) is directed to a method for site-directed mutagenesis of a nucleic acid molecule comprising the steps of :

a) hybridizing a mutagenic oligonucleotide to a target region of a double-stranded nucleic acid molecule, wherein the mutagenic oligonucleotide comprises a mutagen incorporated into a single-stranded nucleic acid that forms a triple-stranded nucleic acid molecule with the target region; and

b) mutating the double-stranded nucleic acid molecule.

Limitations directed to the *in vivo*, *ex vivo*, and *in vitro* applications of the above method have been continually read into the claims by the Examiner but are not in fact present in the claim language. There is no argument that the claims are enabled for at a minimum delivery in vitro to either a cell free system or to isolated cells. While it is the appellants' assertion that such limitations should not be read into any of the claims on appeal, the appellants respectfully submit that the Examiner's assertions relating to the levels of unpredictability with regard to *in vivo* applications for methods are simply not true.

As explicitly discussed in the specification at pages 23-31, monkey COS cells, provide an *in vivo* system by which site-specific triplex formation can be shown to be efficacious. In this system, psoralen is targeted with the triplex-forming oligonucleotides to the targeted site in SV40 DNA (harbored in the monkey cell system). UVA irradiation was used to activate the psoralen to form adducts at that site. Replication of the viral genomes in the monkey cells fixed the adducts into mutations.

Mouse fibroblasts were used to show the ***base-pair specific mutagenesis*** of the *supF* gene, using sequence-specific triplex formation and thereby delivery of the psoralen to that base pair site (see Example 5). Figure 9 clearly shows a dose-response relationship in the induction of *supF* mutations, with a higher frequency seen as the concentration of oligonucleotide to which the cells were exposed was increased. The exact targeted base pair was mutated as intended, thereby illustrating that the use of oligonucleotides linked to psoralen, for example, can target mutations to one specific base pair out of the entire genome of a living mammalian cell.

Table 1 of the Declaration provides further evidence of the *in vivo* applicability of the claimed method, Table 1 illustrates the average mutation frequencies in liver, kidney, skin, colon, small intestine, and lung, as a result of the triplex forming oligonucleotides binding to the polypurine site in supFG1 (AG30) in each of these tissues. These results show that *specific* mutation frequencies *in vivo* were significantly higher in those tissues as compared to tissues derived from the control treated mice. The appellants have addressed and eliminated the notion that the induced mutagenesis obtained from the AG30 treated animals may have resulted from a non-specific effect. This is based upon the lambda cII gene mutation reporter *that showed no induction of mutagenesis* in animals treated with either AG30 or the negative control oligonucleotide (SCR30) as compared with background levels (see Table 2 of the Declaration). One of ordinary skill in the art will certainly agree that these results are *completely consistent* with a gene specific, triplex mediated effect of AG30 on the supFG1 gene of mice (*in vivo* ***site directed*** mutagenesis).

Using the methods and reagents provided in the specification as originally filed, the appellant administered intraperitoneal injections of two different triplex forming oligonucleotides to mice (as described in the Declaration). *Mutagenic analysis on collected tissues confirmed an oligonucleotide-specific induction of mutagenesis in these mice.* "These data indicate efficient tissue uptake and distribution of oligonucleotides in mice after i.p. injections" (see page 9 of Declaration). The Examiner asserted that the quantity of experimentation is undue, on the alleged basis that there exists a number of parameters which would have to be studied to apply this technology to *in vivo* methods. It should be pointed out

that each of (a) the ability of the oligonucleotide to specifically bind the target gene, (b) formation of a stable complex between the oligonucleotide and the target gene, (c) uptake of the oligonucleotide by the cell, and (d) solubility of the oligonucleotide in the cell, have all been addressed in the previously submitted declaration (Glazer Declaration) as well as the specification as originally filed. No experimentation is required!

The appellant did not provide the Glazer Declaration to show what was known at the time of filing. Instead, the appellant submitted the Declaration to show that the methods, reagents, compositions, and art, *as already described and disclosed in the present application,* clearly enable the claims on Appeal. In the Advisory action mailed on May 27, 2003, the Examiner maintains that the "applicant may not use later filed references to demonstrate enablement. This is simply not true in view of the legal standard as set forth above (see point ii). The ***ability*** of those of skill in the art at the time of filing and evidence to prove that statements made in the application **may be submitted for consideration, regardless of when it was developed.**

In view of the foregoing discussion, the appellants respectfully submit that the Board of Appeals review the specification *in view of the Declaration and references cited therein.* The application, as filed, clearly teaches how to make and use the claimed methods. Practicing the claimed methods would not be considered undue in view of the specification as originally filed. The previously submitted Glazer Declaration lends further support to this assertion.

(c) The Examiner has failed to individually examine the independent claims.

It is well established that each claim must be separately examined for patentability. It is not enough, as here, to look at a single independent claim and reject all claims. No rationale has

been presented as to why the subject matter of claims 7-9, directed to the types of mutagen to be incorporated into the single stranded nucleic acid, are not enabled. No rationale has been presented as to why the subject matter of claims 10-14, directed to the type of target region to be affected, are not enabled. Claims 10-14 are directed to specific double-stranded nucleic acid molecules (genes or all or portions of a viral genome).

These claims must be considered separately because each group contains different elements (i.e., the mutagens, and the target sequence to be mutated). The issues are different with regard to enablement of a method to specifically mutate a target site *via* different mutagens and the way in which they are delivered to the specific target site. As discussed throughout this Appeal Brief, it is the oligonucleotide that gives direction/specificity to the mutagen. The target (i.e. genes or all or portions of a viral genome) confer variability to the oligonucleotide, and therefore (claims 10-14) should be recognized for separate consideration.

(9) SUMMARY AND CONCLUSION

As discussed above, the claimed invention is **not** the discovery of mutagens, but rather a method for specifically mutating a target molecule through the administration of a mutagenic oligonucleotide which forms a triplex structure with the target molecule. The specification contains several examples showing that site-specific mutagenesis is achieved not only in cell-free systems but mammalian cells (COS and fibroblast cells). The declaration of Dr. Glazers demonstrates the truth of the statements made in the specification, showing *in vivo* experimental results that are *completely consistent* with a gene specific, triplex mediated effect of AG30 on the supFG1 gene of mice (*in vivo* ***site directed*** mutagenesis). At the very least, the burden has been

shifted to the Patent Office to provide *convincing* evidence or reasoning as to why one could not use the claimed methods of mutagenesis. The rejection merely notes areas of alleged unpredictability and then concludes that the claimed method could not be used and/or that Appellants have failed to demonstrate that the claimed method can be used as disclosed. This later conclusion is improper since Appellants are not required to provide such a demonstration in the absence of a *prima facie* showing of lack of enablement. However, assuming, *arguendo*, that a *prima facie* case has been presented, Appellants have provided ample evidence to show that the state of the art in the field of mutagenesis at the time of filing the Application was such that one skilled in the art would find the specification fully enabling. The Patent Office is required to accept the truth of the Appellants' statements unless a reason to do otherwise can be substantiated. *In re Marzocchi,* 439 F.2d at 223 (CCPA 1971). The Examiner has failed to substantiate any reason why the evidence presented by the Appellants in the specification and the publications does not sufficiently overcome the Examiner's concerns.

The Examiner's assertion that the Glazer Declaration is not based on the specification as filed, is wrong (see page 2 of the Advisory Action mailed on May 27, 2003). Both the Declaration and application as filed rely on 1) polypyrimidine or polypurine regions of DNA to target/bind (see point 6 of the Declaration; and page 10, lines 35-37, of the specification, wherein the base composition of the oligonucleotide is preferred to be homopurine or homopyrimidine thereby binding/targeting polypyrimidine or polypuring regions); and 2) a mutagen that may be attached to an oligonucleotide (see pages 11 and 12 of specification; and the Declaration as a whole). The specification teaches psoralen-linked triplex-forming oligonucleotide targeted

mutagenesis of the *supF* gene in lambda phage (Example 1), targeted mutagenesis in SV40 DNA (Example 4), and targeted mutagenesis in mouse fibroblasts (Example 5); 4) methods of use and administration are clearly detailed on pages 12-14 of the specification. The Examiner's assertion that the Declaration fails to correlate to the specification is rendered moot upon a closer examination of the specification, as outlined above. *Nothing more than what was disclosed in the specification (including knowledge of one of ordinary skill in the art at the time of filing the application) went into the procedures used to generate the results discussed in the Declaration.* The Declaration supports the appellants assertion that the written description enables any person skilled in the art to practice the claims on Appeal.

For the foregoing reasons, Appellant submits that the claims 6-14 are patentable.

Respectfully submitted,

Todd S. Hofmeister
Reg. No. 53,029

Date: June 16, 2003

HOLLAND & KNIGHT LLP
One Atlantic Center, Suite 2000
1201 West Peachtree Street
Atlanta, Georgia 30309-3400
(404) 817-8473
(404) 817-8588 (fax)

Filed: February 14, 2001
APPEAL BRIEF

Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Aisha Wyatt

Date: June 16, 2003

Appendix: Claims On Appeal

6. (original) A method for site-directed mutagenesis of a nucleic acid molecule comprising the steps of :

a) hybridizing a mutagenic oligonucleotide to a target region of a double-stranded nucleic acid molecule, wherein the mutagenic oligonucleotide comprises a mutagen incorporated into a single-stranded nucleic acid that forms a triple-stranded nucleic acid molecule with the target region; and

b) mutating the double-stranded nucleic acid molecule.

7. (original) The method of claim 6 comprising the additional step of activating the mutagen prior to the mutation step.

8. (original) The method of claim 6 wherein the mutagen is selected from the group consisting of psoralen and acridine orange and is activated by light.

9. (original) The method of claim 6 wherein the mutagen is selected from the group consisting of acridine orange, an alkylating agent, a cis-platinum analog, a hematoporphyrin, a hematoporphyrin derivative, mitomycin C, a radionuclide, and a molecule that interacts with radiation to become mutagenic.

10. (original) The method of claim 6 wherein the mutation alters the activity of the double-stranded nucleic acid molecule.

11. (original) The method of claim 6 wherein the double-stranded nucleic acid molecule is a gene.

12. (original) The method of claim 6 wherein the gene is an oncogene.

13. (original) The method of claim 6 wherein the gene is a defective gene.

14. (original) The method of claim 6 wherein the double-stranded nucleic acid molecule is all or a portion of a viral genome.

TABLE OF CONTENTS